Expression of Ki67 was 11% when 1/3 of the cells were spared but augmented till 36% when only 10% were spared.

The viability ranged between 93-98% with the highest in the 1/10 subcultivation group and the lowest in the 1/3 subcultivation group.

Conclusion: We could thus demonstrate a remarkable ability of this cell line to adapt to changing conditions. A decrease in doubling time up to 50 % due to reducing number of cells in each cell culture dish at subcultivation was seen. This accelerated proliferation seems to mainly have been accomplished by recruiting a higher fraction of cells in proliferation reflected by the increased Ki67 expression.

An accelerated proliferation might be of importance clinically, for example considering the effect of fractionated irradiation in a radiosensitive cell population.

P2-123 **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

Induction of apoptosis by rhein via reactive oxygen species production, GADD153 expression, and caspase-3 activation in human lung cancer A-549 cells

Hsia, Te-Chun[1] Hang, Liang-Wen[1] Chung, Jing-Gung[2]

[1] China Medical University Hospital, Taichung, Taiwan [2] Department of Biological Science and Technology, China Medical University, Taichung, Taiwan

Background: Although rhein had been shown to induce apoptosis in several cancer cell lines, the action mechanism of rhein induced cell cycle arrest and apoptosis at the molecular level is not well known.

Method: In this study, we use flow cytometry analysis of DNA content for cell cycle and apoptosis from A-549 cells treated with different concentrations of rhein; Poly (ADP ribose) monoclonal antibody assay for apoptosis in A-549 cells after treated with rhein; inhibition of rhein-induced apoptosis by the caspase-3 inhibitor Ac-DEVD-CHO in A-549 cells etc. We investigated the mechanisms of rhein on a human lung cancer A-549 cell that induce G0/G1 phase arrest and ROS and Ca2+ productions that play an important role for apoptosis which are characterized by caspase activation and mitochondria dependent pathway. Rhein induced G0/G1 arrest through inhibition of cyclin D3, Cdk4, Cdk4, and Cyclin E.

Result: The efficacious induction of apoptosis was observed at 50 μM for 12 h and up to 72 h examinations which were examined by flow cytometric method. Flow cytometric analysis demonstrated that rhein induced the loss of mitochondrial membrane potential (Δ m), cytochrome c release from mitochondrion, promoted capases-9 activation then promoted the activation of caspase-3 and led to apoptosis. Rhein also increased the levels of Gadd153, p53, p21, Bax and cytochrome c but decreased the levels of Bcl-2. The Ca2+ chelator BAPTA was added to the cells before rhein was added to the cells, and it blocked the Ca2+ production and also inhibited rhein-induced apoptosis in A-549 cells.

Conclusions: Our data demonstrated that rhein induces apoptosis in A-549 cells via a Ca2+-dependent mitochondrial death pathway.

P2-124 **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

Focal adhesion paxillin induces nodular cell growth, invasion, and angiogenesis in lung cancer

Jagadeeswaran, Ramasamy; Zumba, Osvaldo; Krishnaswamy, Soundararajan; Janamanchi, Varalakshmi; Seiwert, Tanguy Y.; Loganathan, Sivakumar; Kanteti, Rajani; Nallasura, Vidya; Faoro, Leonardo; Vokes, Everett E.; Salgia, Ravi

University of Chicago, Chicago, IL, USA

Lung cancer is characterized by abnormal cell growth, invasion, and angiogenesis. The actin cytoskeleton plays a major role in these processes. In lung cancer tissues, but not other tumors, we have found that paxillin can be overexpressed, amplified, or mutated. In vivo properties of mutant paxillin (A127T) and wild type paxillin-expressing cells in nude mice were determined utilizing H522 NSCLC cell line in a mouse xenograft model.H522 has no significant expression of paxillin,therefore the properties of paxillin-transfected cells could be studied. Tumor growth in the A127T mutant paxillin markedly exceeded ($P = 0.0019$) that in the control vector or wild-type paxillin. H522 cells grew in nude mice as a solid mass without any invasion or angiogenesis, whereas both wild-type paxillin and the A127T paxillin mutant expressing H522 cells grew as nodular tumors. In addition to nodularity, A127T paxillin expressing H522 xenograft tumors were highly invasive into the adjacent muscle tissue. Upon gross examination, the A127T paxillin tumors had larger nodules as compared to the wild-type paxillin tumors. Tumor sections from mice in various groups were examined using antibodies specific for nuclear antigen Ki-67 (a marker for active cell division), CD31 (measuring microvessel density, MVD) and VEGF (vascular endothelial growth factor). There was enhanced cell proliferation, increased stroma, increased MVD and increased VEGF expression in wild type paxillin H522 xenografts. In the A127T paxillin H522 xenografts, as compared to paxillin negative or wild-type paxillin positive cells, there was enhanced cell proliferation with decreased stroma formation, MVD, and VEGF. There was a strong correlation between paxillin expression and angiogenesis ($p=0.01$). In conclusion, results from this study establish an important role for paxillin in lung cancer.

P2-125 **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

Amplification of c-Met in a subset of lung cancer cells leads to activation of m-Tor pathway

Jagadeeswaran, Ramasamy; Zumba, Osvaldo; Salgia, Ravi

University of Chicago, Chicago, IL, USA

Non-small cell lung cancer (NSCLC) is a difficult disease to treat. Even with the best therapies and the recent advent of novel molecularly targeted therapies, the overall survival for all NSCLC patients is only 20% over a five year period. c-Met receptor tyrosine kinases (RTKs) have been shown to be important in a variety of malignancies. We have examined the expression and gene amplification of c-Met receptor in various NSCLC cell lines using standard immunoblotting and FISH analysis respectively. It was found that there was overexpression of c-Met in most of the NSCLC cell lines (~75%), including H441, SKLU-1, H1993, A549, H1838, H358 and SW1573 except H-522 and H661. This was also evident in the lung tumor tissue immunoblotting and paraffin embedded lung cancer tissue micro array. FISH analysis revealed amplification of the c-Met region on chromosome 7p11.2 to copy number 15 in 22% (two out of nine) of NSCLC cell lines, and correlated with high expression. Constitutively activated c-Met was

found in the amplified cell lines. This amplification confers sensitivity of NSCLC to inhibitors of the tyrosine kinase activity of c-Met. To further determine the molecular characteristics of cell lines carrying c-Met amplification, we investigated the levels of downstream effectors of c-Met, such as phospho paxillin, phospho-extracellular signal-regulated kinase and phospho-S6 proteins. Interestingly, S6 kinase a serine-threonine kinase whose activation is thought to regulate a wide array of cellular processes involved in the mitogenic response including protein synthesis, translation of specific mRNA species, and cell cycle progression from G1 to S phase was more frequently activated in cell lines with gene amplification of c-Met than in their wild-type counterparts. c- Met amplification may thus identify a subset of lung cancers that are uniquely altered signaling which potentially sensitive to c-Met inhibition.

P2-126 **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

The role of pax transcription factors in lung carcinogenesis: relationship to c-Met receptor tyrosine kinase

Kanteti, Rajani; Nallasura, Vidya; Wang, Cindy; Hasan, Rabia; Loganathan, Sivakumar; Jagadeeswaran, Ramasamy; Husain, Aliya; Lang, Deborah; Salgia, Ravi

University of Chicago, Chicago, IL, USA

Background: Lung cancer still remains one of the leading causes of all cancer related deaths. They can be divided into two major types, non small cell (NSCLC) and small cell (SCLC) lung cancers. Amongst the two, prognosis in patients with NSCLC is better compared to those with SCLC, however the overall survival rate is still very low (17%). A plethora of molecular changes due to various genomic alterations are known to contribute to the development and spread of lung cancers, for instance mutations in p53, RB, Ras, several receptor and non-receptor tyrosine kinases are known to contribute to the variety of phenotypes seen in lung cancer. One of the long term goals of our lab is to map and study the biological significance of loss and gain of function mutations in various signaling molecules in lung cancer; with the idea of developing novel therapeutics. In this regard, we examined the role of Pax transcription factors in lung cancer. To date, the Pax family consists of nine members that are all characterized by the presence of a paired domain. They are indispensable for various developmental processes and several of them are known to play a significant role in the development of various cancers. In this initial study, we used a panel of both NSCLC and SCLC cell lines to determine the relative levels of various Pax proteins.

Methods: Whole cell lysates from a panel of NSCLC and SCLC cell lines were prepared using RIPA Buffer. Equivalent amount cell lysates proteins were separated by SDS-PAGE and subjected to immunoblotting using various commercially available anti-Pax antibodies.

Specific Pax knockdown cells were also generated by transfecting commercially available siRNA. The loss of the particular Pax expression was determined using immunoblotting procedures.

Results: We detected significant expression of Pax8 in NSCLC and Pax5 in SCLC cell lines. In addition we could detect BCL2 but not BCL-XL, especially in the SCLC cell lysates. Since Pax3 is a known direct transcriptional activator of c-Met, a receptor tyrosine kinase that is known to play a significant role in cancer metastasis, we therefore determined the protein expression levels of c-Met in the above cell lines. In general, in most of the cell lines where Pax expression was detected, we also detected comparable levels of c-MET. We are currently determining how the Pax factors can regulate biological and biochemical processes in lung cancer.

Conclusions: Differential expression of Pax8 in NSCLC and Pax5 in SCLC can be further tested as biomarkers to characterize lung tumor tissue biopsies. This differential expression can be combined with other biomarkers to help distinguish between SCLC, NSCLC, and other tumors.

P2-127 **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

Effects of insulin like growth factor-1 on repair mechanism of DNA damage induced by cis-Diammineplatinum dichloride in NSCLC

Jeon, Jeong Hee[1] Chang, Yoon Soo[2] Kim, Se Kyu[2] Kim, Hyung Jung[2] Chang, Joon[2] Ahn, Chul Min[2] Kim, Sung Kyu[2]

[1] Brain Korea 21 Project for Medical Sciences on Yonsei University college of Medicine, Seoul, Seoul, Korea [2] Department of internal Medicine on Yonsei University college of Medicine, Seoul, Seoul, Korea

Propose: Authors are to investigate DNA repair mechanisms involved in DNA damage induced by cis-Diammineplatinum dichloride(cisplatin), which is widely used for treatment of NSCLC. We also studied relevance of IGF-1 system in DNA repair mechanisms.

Methods: The effect on non-small cell lung cancer (NSCLC) cell line NCI-H1299 and NCI-H460 proliferation by IGF-1 and cisplatin treatment was measured by MTT assay. Changes of molecular system consisting HRR and NEJH were evaluated by immunoblotting and immunocytochemistry. Comet assay was applied to analyze influence of IGF-1 on DNA damage repair.

Results:

1. Cisplatin treatment resulted in inhibition of cell proliferation in a dose dependent manner. IC50 and IC80 are about 33.3 uM and 9.1 uM in H1299 and 33.7uM and 8uM in H460 cells. 50 ng/ml IGF-1 treatment on each cells recovered about 20% of cell proliferation repressed by cisplatin.
2. Immunocytochemical study showed cisplatin treatment induced activation of gamma H2AX, and addition of 50 ng/mL IGF-1 potentated its activation. Nuclear translocation of ATM and IRS-1 was promoted by cisplatin treatment, but was suppressed by IGF-1. On the other hand, translocation of ATR was enhanced by cisplatin and facilitated by IGF-1 treatment.
3. Phsphorylation of ATM induced by cisplatin was confirmed by immunoblotting, but not with IGF-1. Whereas IGF-1 induced ATR activation, and promoted gamma H2AX formation.
4. Reduced cisplatin induced DNA damage could verify IGF-1 effect with comet assay.

Conclusions: Activation of ATR pathway by IGF-1 might be primary recovery mechanism of cisplatin induced DNA damage.

P2-128 **BSTB: Tumor and Cell Biology Posters, Tue, Sept 4**

Sequence dependant antiproliferative effect of cytotoxic drugs and epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI) in non-small cell lung cancer cell lines (NCI-H1975).

Kim, Kyu-Sik[1] Jeong, Ju-Yeon[1] Kim, Mi-Ran[1] Lim, Jung-Hwan[1] Chae, Dong-Ryeol[1] Cho, Gye-Jung[1] Ju, Jin-Young[1] Oh, In-Jae[1] Kim, Yu-Il[1] Lim, Sung-Chul[1] Kim, Young-Chul[1] Park, Kyung-Ok[2]

[1] Chonnam National University Hospital, Gwangju, Korea [2] Ilgok Hospital